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ARMY MEDICAL RESEARCH INST OF INFECTIOUS DISEASES FR--ETC F/G 6/5
SYSTEMIC METABOLIC ALTERATIONS ASSOCIATED WITH REPEATED INJECTI--ETC(U)
JAN 77 M C POWANDA, M L SAMMONS, E L STEPHEN

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Polyribonucleoside acid-polyribocytidylic acid complexed with poly-L-lysine [poly (ICLC)] injected intramuscularly into rats (0.3 or 3.0 mg/kg) produces fever, altered white blood cell count, a slight depression in plasma zinc, increased amino acid uptake into liver and a two-to three-fold increase in plasma acute-plasma globulins. It is suggested that these systemic metabolic alterations may be responsible for some of the nonspecific protective effects of members of this family of compounds and may have to be taken into consideration when poly(ICLC) is used in therapy.

**Systemic Metabolic Alterations Associated With Repeated Injections of a
Modified Polyribonucleosinic-Polyribocytidylic Acid Complex**

Michael C. Powanda, Myrl L. Sammons, and Edward L. Stephen

Running head: Poly (ICLC)-Induced Metabolic Alterations

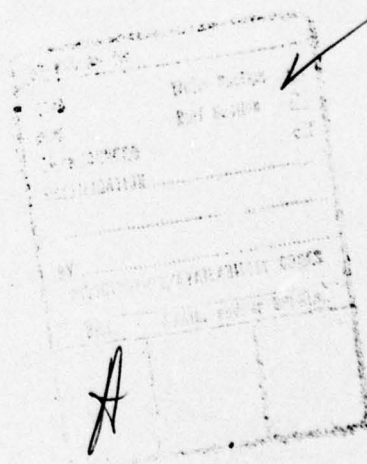
From the United States Army Medical Research Institute of Infectious
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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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Abstract

Polyribonucleosinic acid-polyribocytidylic acid complexed with poly-L-lysine [poly (ICLC)] injected intramuscularly into rats (0.3 or 3.0 mg/kg) produces fever, altered white blood cell count, a slight depression in plasma zinc, increased amino acid uptake into liver and a two-to three-fold increase in plasma acute-plasma globulins. It is suggested that these systemic metabolic alterations may be responsible for some of the nonspecific protective effects of members of this family of compounds and may have to be taken into consideration when poly(ICLC) is used in therapy.



Polyribonucleosinic-polycytidylic acid complexes [poly(I)·poly(C)] are potent inducers of interferon in rodents and rabbits [1,2]. In primates, however, these compounds are poor interferon inducers, apparently because the complex is rapidly degraded by plasma nucleases [3]. A modified, nuclease resistant form of poly(I)·poly(C) has therefore been developed for use in man. This modified poly(I)·poly(ICLC), induces interferon in chimpanzees and rhesus and cynomolgus monkeys [4, 5]. Poly(I)·poly(C) itself, in addition to inducing interferon, also causes fever [3, 6] and depressions in plasma zinc [7]. Fever is presumably produced as a result of poly(I)·poly(C)-induced release of endogenous pyrogen from leukocytes [3]. A decrease in plasma zinc can be elicited by other factors derived from leukocytes [8]. Under certain conditions these leukocyte-derived factors can also stimulate the uptake of amino acids by the liver and produce increases in plasma acute-phase globulins in experimental animals [8, 9]. The following study was conducted to ascertain whether poly(ICLC) which is intended for use in man, would alter various aspects of trace metal, amino acid and protein metabolism. Such information would be of value since diverse and profound alterations in plasma zinc, copper, amino acid and acute-phase globulin concentrations occur during many viral illnesses [10] and thus the metabolic effects of the disease and those of the therapy might well be confused.

Materials and Methods

Male, Fisher-Dunning rats, weighing approximately 150-175 g were supplied by Microbiological Associates (Walkersville, Md.). The rats were maintained in light- and temperature-controlled rooms (12 hr light and 12 hr dark, 25C) for one week before use. They were fed Purina pellets *ad lib.* until the initiation of the experiment at which time food was withheld from all animals to obviate any differences that might arise from drug induced anorexia. Twenty-four hours prior to the injection of poly(ICLC) or saline, the rats were injected *sc* with 1 μ Ci of [14 C]aminoisobutyric acid (AIB)/100 g body weight (New England Nuclear, Boston, Mass.).

The stock poly(ICLC) solution (lot CC-69-A) contained 2 mg poly(I)·poly(C), 1.5 mg poly-L-lysine, 0.5 mg carboxymethylcellulose/ml and was supplied by Dr. H. B. Levy, NIAID, NIH, Bethesda, Md. Poly(ICLC) or saline for the controls was administered *im* according to the schedule outlined in table 1. At selected times after poly(ICLC) injections, groups of eight rats were weighed, rectal temperature obtained (Yellow Springs telethermometer) and then were exsanguinated under halothane anesthesia by transecting the vena cava and collecting the blood from the pleural cavity. Heparin (100 units/10 ml) was used in collecting the blood. A blood sample was taken to determine total white blood cell count, the differential and hematocrit. The whole liver was removed, weighed and perfused with 0.14 N saline. A 1-g portion of the liver was taken for assessment of [14 C] AIB uptake [11]. The spleen was also removed and weighed.

Plasma was analyzed for glucose [12], zinc and copper [13], haptoglobin [14], seromucoid [15], α_2 macrofetoprotein [16], albumin and transferrin [17], and free fatty acids [18].

Results

Poly(ICLC) administered iv in doses of 0.3 or 3.0 mg/kg, is a potent inducer of interferon in monkeys (100-1,000 units/ml) [5]. Poly(ICLC) at both the 0.3 and 3.0 mg/kg level elicited a febrile response in rats 6 hr following the first injection, but only the higher dose consistently provoked a significant hyperthermia upon subsequent injections (table 2). The higher dose of poly(ICLC) produced mild hepatosplenomegaly while 0.3 mg only caused transitory hepatomegaly (table 2). Both doses of poly(ICLC) initially provoked alterations in white blood cell count and distribution, but by the third injection these changes had all but disappeared (figure 1).

Neither level of poly(ICLC) resulted in a significant alteration in plasma glucose concentration (table 2). Both doses caused an initial increase in plasma free fatty acids and sporadic decreases thereafter (table 2). Both 0.3 and 3.0 mg/kg poly(ICLC) occasioned small, albeit significant, decreases in plasma zinc, but no significant change in plasma copper concentration (table 2). Both concentrations of poly(ICLC) provoked an increased uptake of [^{14}C]AIB by the liver suggesting increased uptake of amino acids in general (figure 2).

Poly(ICLC) elicited an increase in plasma albumin within 6 hr after the first injection but thereafter small but significant decreases were in evidence (figure 3). A similar pattern was observed in regard to transferrin (figure 4). Seromucoid, in contrast, was significantly

increased within 6 hr following the initial injection of 3.0 mg/kg. Eventually both doses of the drug resulted in plasma levels of seromucoid which were two to three times that of controls (figure 5). Plasma haptoglobin was significantly increased 24 hr after either dose of poly(ICLC). A second injection of poly(ICLC) evoked no further increase but the third injection appeared to cause a dose-dependent enhancement (figure 6). Alpha₂-macroglobulin, an index of trauma/infection, was only minimally evident in the plasma even after three injections of poly(ICLC) (table 2).

Discussion

Repeated im injections of a modified polyribonucleic-polyribocytidylic acid complex intended for use as an antiviral agent in man resulted in significantly increased amino acid uptake into liver, a two to three fold increase in plasma acute phase globulin concentration and a 15-35% decrease in plasma zinc concentration in rats. Many of these alterations in systemic metabolism are akin to those observed during infection. A 25-50% decrease in plasma zinc has been observed during numerous acute infections in both men and animals [10]. A decrease of similar magnitude can be produced in rats by ip [8, 9] or intracerebroventricular [19] injection of leukocyte-derived factors and also occurs in response to in vivo phagocytosis of killed organisms [20]. Increased amino acid uptake by liver, monitored using nonmetabolizable amino acid analogues has been demonstrated in a number of experimental infections and can be elicited by injections of leukocyte-derived factors [9, 21, 22]. A two to four fold increase in seromucoid concentration (indicative of acute-phase globulin synthesis and/or

release) is associated with most infections [10] and can be simulated by the injection of leukocyte-derived factors [9]. Haptoglobin, an acute-phase globulin, is increased in a number of infections [10]; factors derived from both polymorphonuclear leukocytes [9] and macrophages [23] can produce an increase in this protein. It is thus conceivable that poly(ICLC) may produce the alterations in host metabolism detailed in this paper through phagocytic cell activation with the consequent release of endogenous mediators. Certainly poly(I)·poly(C) has been shown to stimulate endogenous pyrogen release from leukocytes in vitro [3] and fever often accompanies both poly(I)·poly(C) and poly(ICLC) administration [3, 5, 6]. It is, however, somewhat surprising that a decrease in plasma zinc occurs following poly(ICLC) administration without an increase in plasma copper, since the latter is often observed during infection [10] and can also be produced by leukocytic endogenous mediator (LEM) [9]. The fact that increased amino acid uptake into liver and increased seromucoid and haptoglobin concentration are observed in the present study with little or no change in α_2 -macroglobulin also requires consideration. But the absence of an increase in plasma copper or the failure of α_2 -macroglobulin to appear do not argue against the role of phagocyte-derived endogenous mediators in poly(ICLC) induced metabolic alterations. Rather the data suggest that even repeated injections of poly(ICLC) provide only a modest stimulus. If such were not the case one would expect substantial decreases in plasma albumin such as are observed during infection [10] or after multiple injections of LEM [24]. Nor were there appreciable alternations in transferrin which might be expected to decrease markedly [10, 17].

Cardiac catheterization with a venous cutdown, presumably only a mildly traumatic procedure, produces increases in certain acute-phase globulins without an appreciable decrease in albumin [25]. Moreover the data are consistent with other evidence of a multiplicity of mediators [26] and may even suggest that there is a differential release of mediators dependent upon the type of phagocytic cell which is stimulated and the mode or degree of stimulation. The fever and slight hepatosplenomegaly are also consistent with reticuloendothelial cell activation.

The initial increase and subsequent decrease in free fatty acids are not presently interpretable in terms of endogenous mediators, although both decreases and increases in free fatty acids are noted during various infections [10] and this may be a direct or indirect effect of such mediators.

It is unlikely that the metabolic alterations seen in rats injected with poly(ICLC) are the result of contamination of the drug with endotoxin since these are only small decreases in plasma zinc despite two to three fold increases in haptoglobin and seromucoid. Plasma zinc concentration in the rat is a very sensitive indicator of the presence of endotoxin, 0.1 μg being sufficient to cause a depression of greater than that observed in the poly(ICLC) treated rats [27], whereas at least 200 μg would be required to provoke an increase in haptoglobin and seromucoid [9]. The absence of a significant change in hematocrit and plasma glucose indicate that the observed changes in plasma constituents are not the result of changes in plasma volume and that poly(ICLC) is selective in its effect on host metabolism.

The above findings indicate that chronic therapy with poly(ICLC) may affect the metabolic state of patients. A priori, these alterations do not seem to connote toxicity but rather indicate that corrective action in the form of trace metal and/or nitrogen supplementation may be required during prolonged poly(ICLC) therapy.

These data also cause one to wonder if the antiviral activity of poly(I)·poly(C) and other interferon inducers may in part be the result of altered host metabolism. Perhaps the ability to alter various aspects of host metabolism, as well as induce interferon, may explain the seemingly nonspecific protection against certain bacteria, fungi and protozoa afforded by a variety of interferon inducers [28-31]. It has been postulated recently that altered trace metal and amino acid distribution and increased acute-phase globulin synthesis are a part of a generalized system of host defense responses designed to minimize tissue destruction associated with phagocytic cell release of lysosomal enzymes, promote granulopoiesis, activate macrophages, and aid in the development of humoral and cellular immunity [32]. If such is the case then a knowledge of the metabolic alterations produced by potential antiviral agents such as poly(ICLC) may help to design better regimens of therapy from theoretical rather than empirical considerations and thus decrease the chance of exacerbating the disease [33, 34].

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Table 1. Study protocol

Day and procedure	Hour	Poly(ICLC) injected		
		3.0 mg/kg	0.3 mg/kg	None, Saline
Day 0				
Inject poly(ICLC)	0	[40]	[40]	[40]
Obtain samples from 8 rats	6	+	+	+
Day 1				
Obtain samples from 8 rats	24	+	+	+
Inject poly(ICLC)	24	[24]	[24]	[24]
Obtain samples from 8 rats	30	+	+	+
Day 2				
Obtain samples from 8 rats	48	+	+	+
Inject poly(ICLC)	48	[8]	[8]	[8]
Obtain samples from 8 rats	54	+	+	+

Note. [] = number of rats in each group receiving injections at each time.

Hour is no. from initiation of study, i.e., first injection of poly(ICLC).

Table 2. Temperature, spleen and liver weights, hematocrit, and other metabolic values.

Time Poly(ICLC) (mg/kg)	Rectal temp.	Spleen wt (mg/100µg)	Liver wt (g/100µg)	HCT % RBC	Glucose (mg/dl)	FFA (µEq/dl)	Zinc (µg/dl)	Copper (µg/dl)	α ₂ -MFP (units/ml)
6 hours									
0	37.0 ± 0	225 ± 8	2.96 ± 0.04	46 ± 1	171 ± 5	585 ± 43	140 ± 4	123 ± 4	0
0.3	38.1 ± 0.2	206 ± 9	3.03 ± 0.06	44 ± 1	170 ± 4	883 ± 78*	125 ± 6	117 ± 3	0
3.0	38.2 ± 0.2	236 ± 8	3.17 ± 0.10	46 ± 1	175 ± 7	831 ± 74*	119 ± 4*	127 ± 8	0
24 hours									
0	37.1 ± 0.2	248 ± 8	2.79 ± 0.03	44 ± 1	137 ± 3	567 ± 46	125 ± 3	136 ± 5	0
0.3	37.7 ± 0.3	239 ± 11	2.85 ± 0.09	47 ± 1	142 ± 9	482 ± 37	109 ± 5*	137 ± 11	0
3.0	37.9 ± 0.2	259 ± 7	3.07 ± 0.05**	46 ± 1	153 ± 5	335 ± 29**	94 ± 4**	134 ± 4	0.6 ± 0.2
30 hours									
0	37.2 ± 0.2	280 ± 10	2.67 ± 0.03	45 ± 1	121 ± 6	549 ± 50	116 ± 5	115 ± 6	0
0.3	37.8 ± 0.1	275 ± 6	2.98 ± 0.05**	46 ± 1	138 ± 3	304 ± 28**	81 ± 3**	117 ± 3	0
3.0	38.7 ± 0.2	260 ± 9	3.23 ± 0.13**	46 ± 1	129 ± 3	392 ± 37	80 ± 3**	127 ± 6	1.9 ± 0.6
48 hours									
0	36.3 ± 0.1	200 ± 8	2.49 ± 0.05	47 ± 1	133 ± 5	453 ± 55	110 ± 3	131 ± 13	0
0.3	36.2 ± 0.1	221 ± 6	2.70 ± 0.04*	46 ± 1	146 ± 3	281 ± 55	105 ± 3	102 ± 5	0.4 ± 0.4
3.0	37.4 ± 0.30*	251 ± 14*	2.78 ± 0.05**	43 ± 1	134 ± 5	373 ± 25	85 ± 3**	148 ± 7	1.8 ± 0.7
54 hours									
0	36.9 ± 0.31	234 ± 7	2.50 ± 0.50	47 ± 1	128 ± 5	551 ± 40	116 ± 2	119 ± 8	0
0.3	37.6 ± 0.31	251 ± 12	2.63 ± 0.05	46 ± 1	135 ± 6	311 ± 26*	92 ± 5**	135 ± 3	0.4 ± 0.2
3.0	37.9 ± 0.11	288 ± 66**	2.93 ± 0.04**	45 ± 1	132 ± 5	215 ± 32**	72 ± 3**	144 ± 5	2.0 ± 0.5

Note. Values are means ± SEM.

*P < 0.01, **P < 0.001 vs saline controls at each time period.

Legend to Figures

Fig 1. White blood cell distribution following repeated injections of poly(ICLC). Significance was assessed vs. saline controls at each time period.

Fig 2. Uptake of [14 C] aminoisobutyric acid by liver following repeated injections of poly(ICLC). Significance was assessed vs. saline controls at each time period.

Fig 3. Plasma albumin concentration following repeated injections of poly(ICLC). Significance was assessed vs. saline controls at each time period.

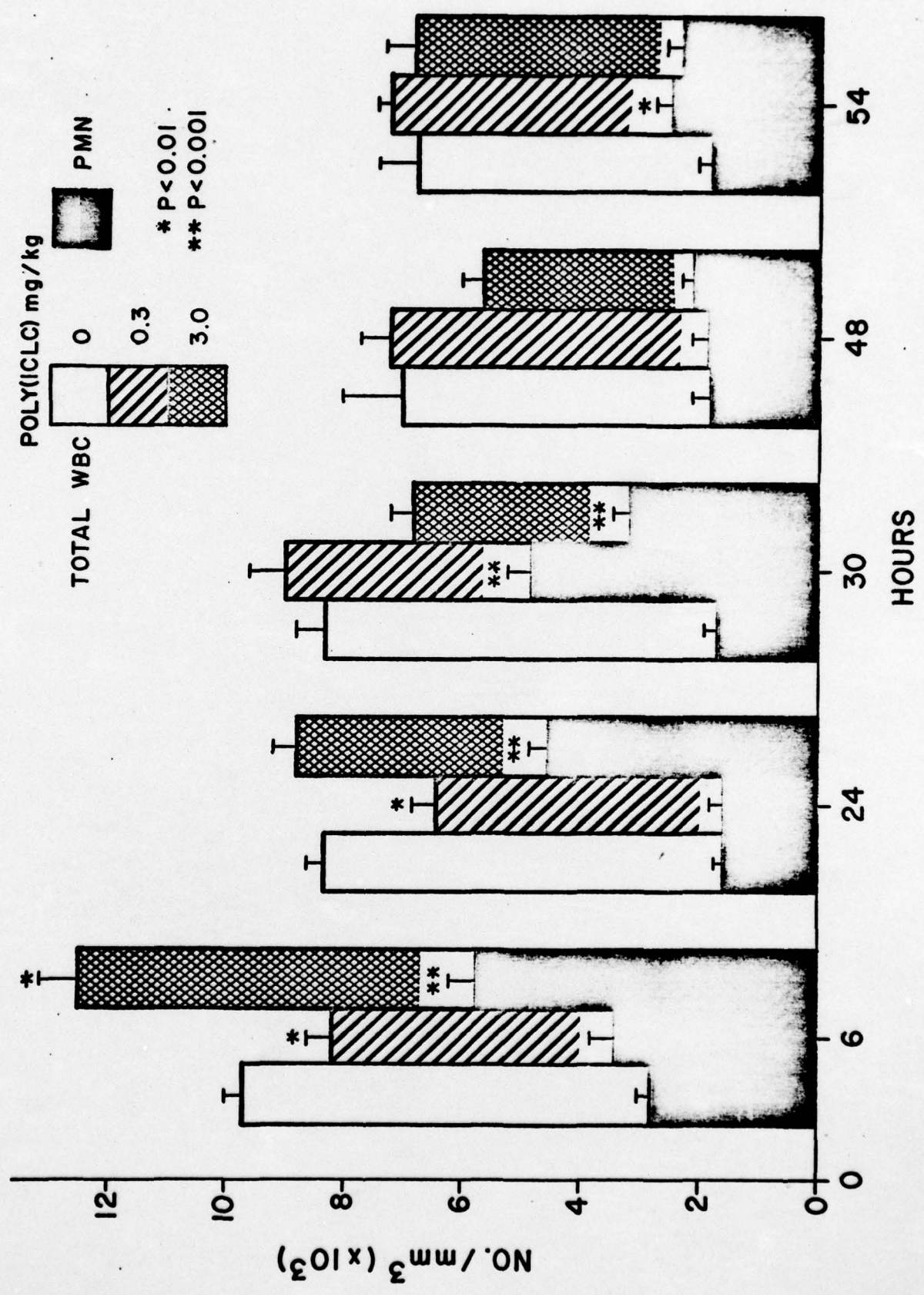
Fig 4. Plasma transferrin concentration following repeated injections of poly(ICLC). Significance was assessed vs. saline controls at each time period.

Fig 5. Plasma seromucoid concentration following repeated injections of poly(ICLC). Significance was assessed vs. saline controls at each time period.

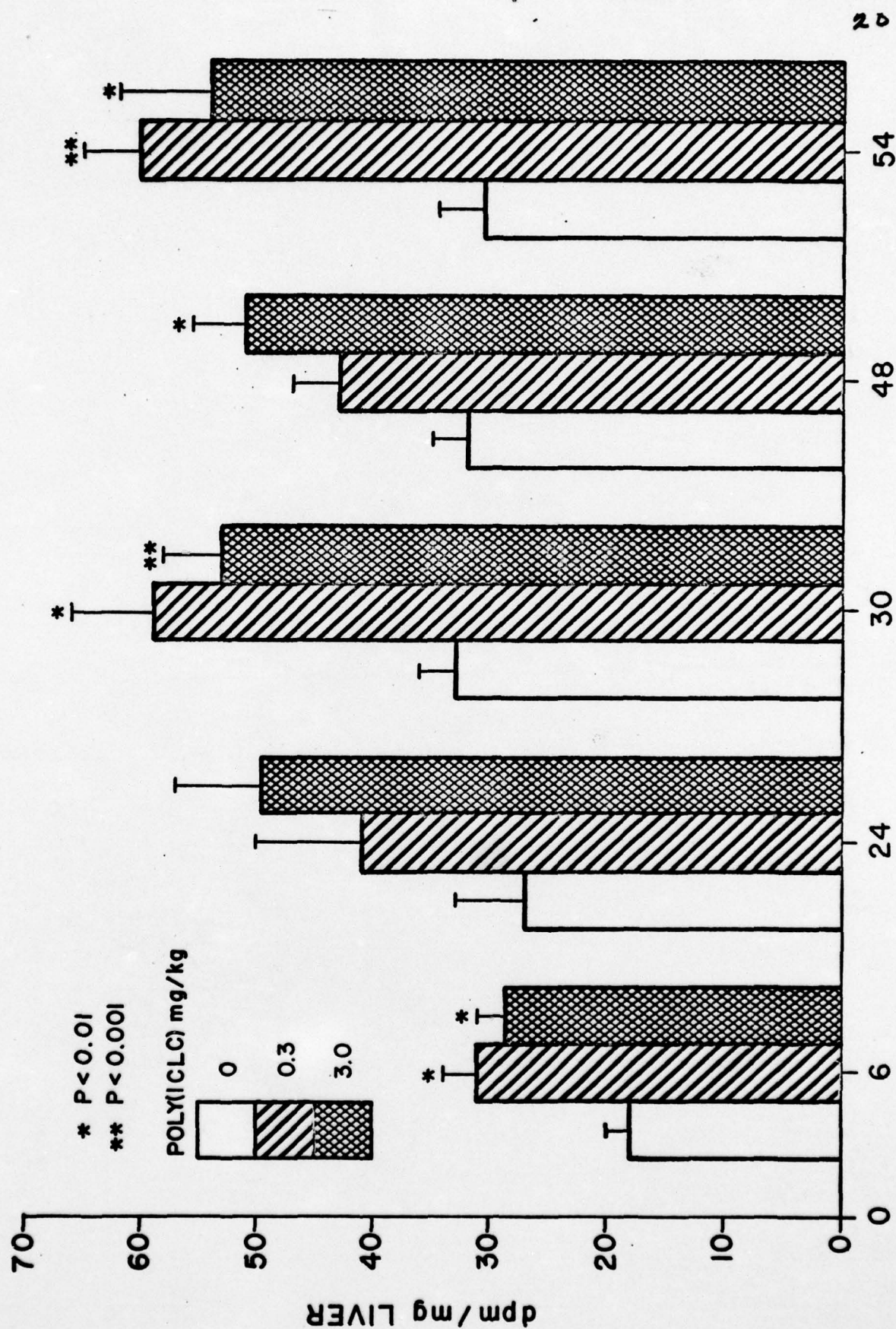
Fig 6. Plasma haptoglobin concentration following repeated injections of poly(ICLC). Significance was assessed vs. saline controls at each time period.

Fig 1.

WHITE BLOOD CELL DISTRIBUTION



HEPATIC [¹⁴C]AIB UPTAKE

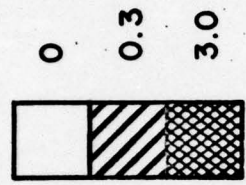


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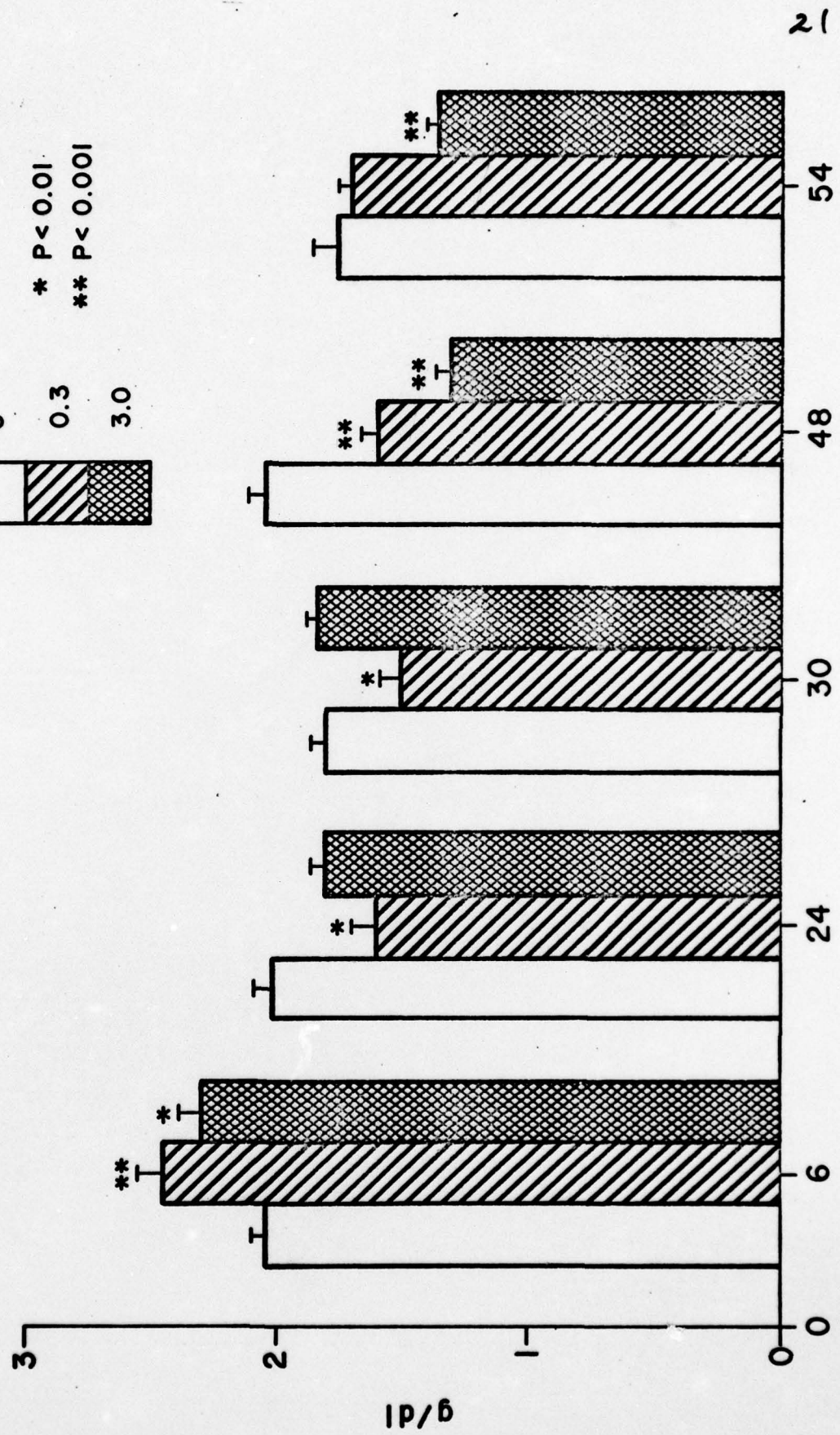
POLY(IGLC)

PLASMA ALBUMIN

POLY(IGLC) mg/kg



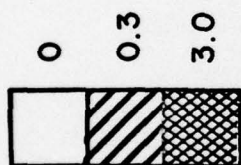
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** $P < 0.001$



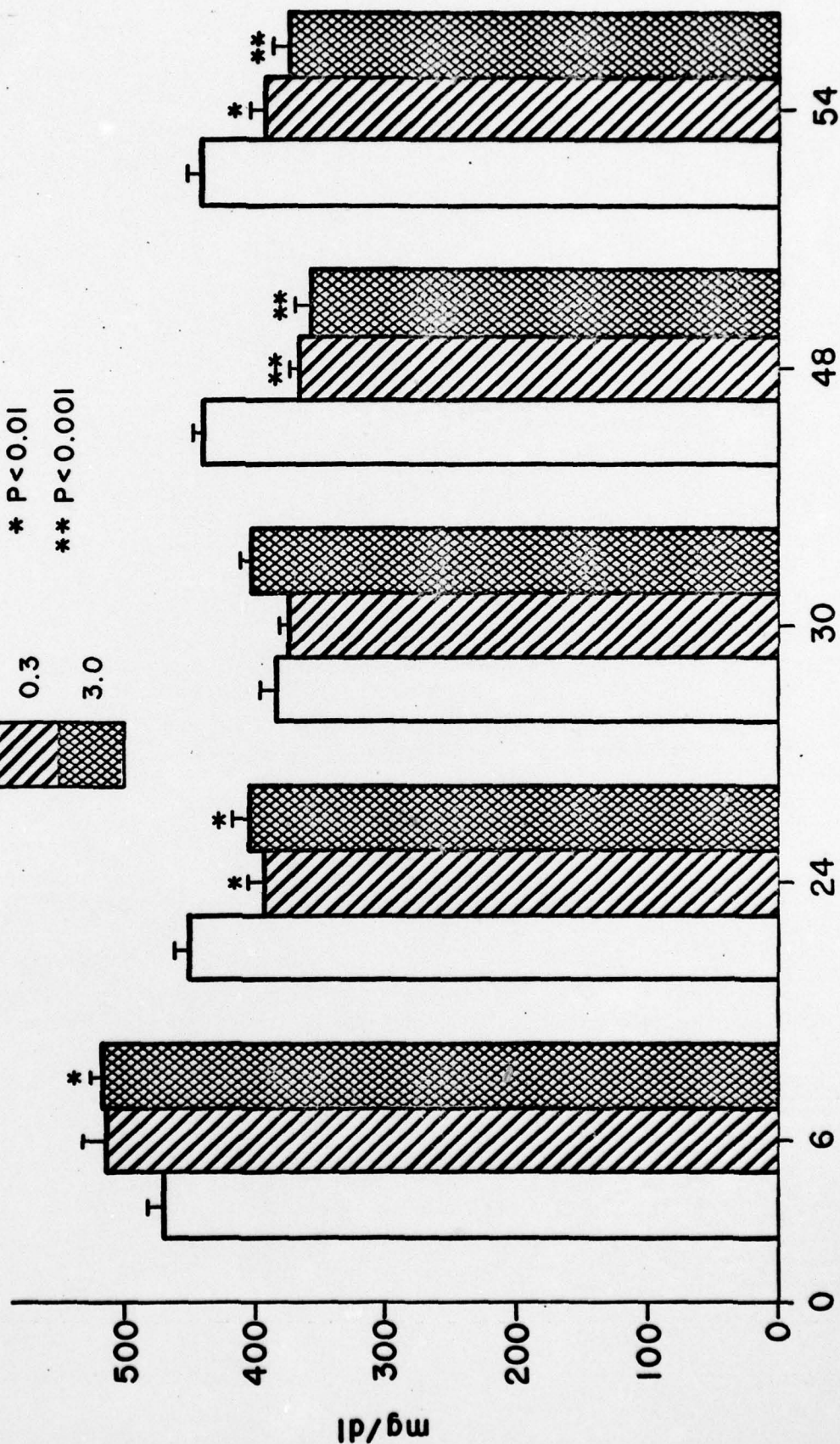
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PLASMA TRANSFERRIN

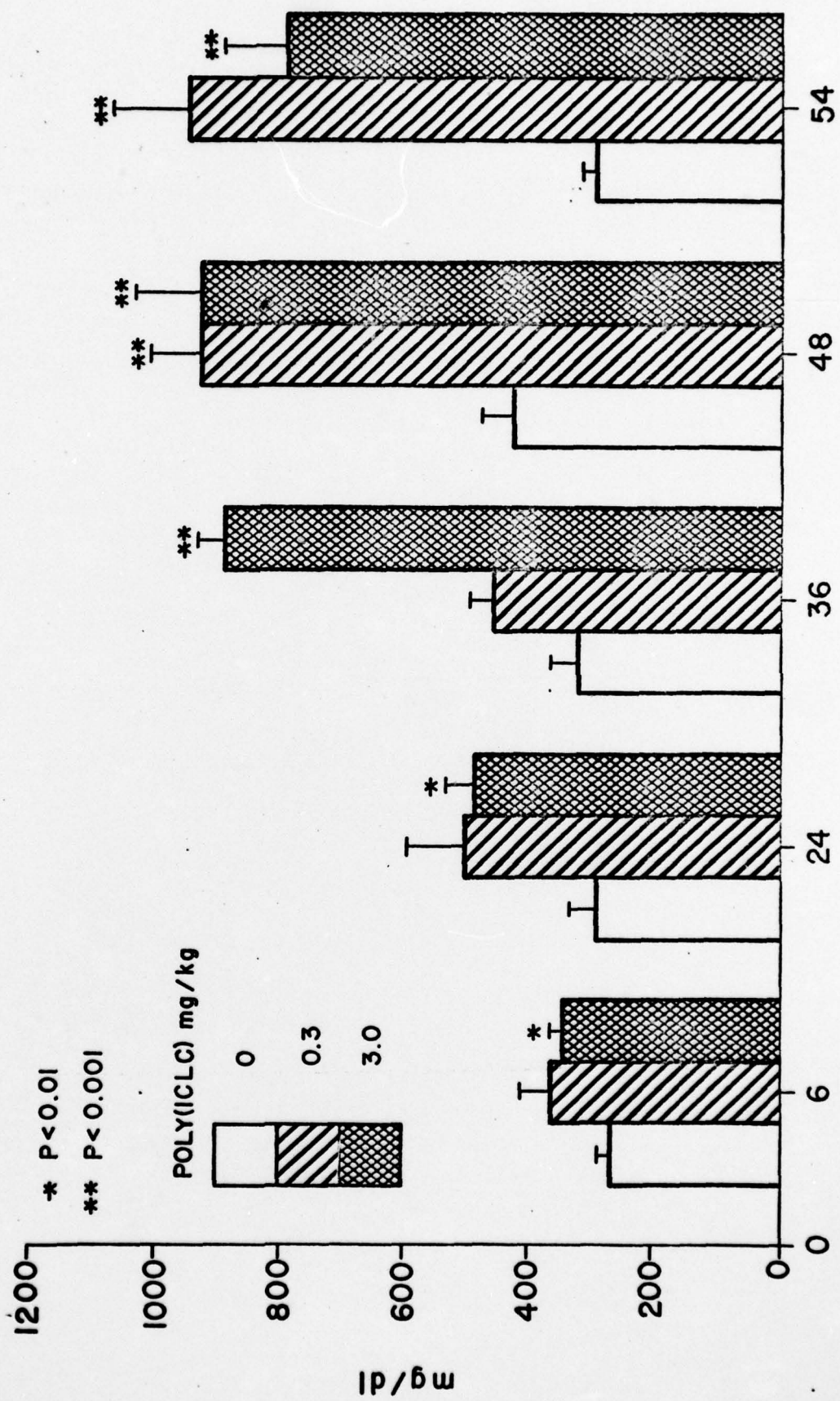
POLY(ICLC) mg/kg



* $P < 0.01$
** $P < 0.001$



SEROMUCOID



HAPTOGLOBIN

